PRODUCTION OF BACTERIAL CELLULOSE FILMS BY *GLUCONOACETOBACTER XYLINUS* FOR LIPASE IMMOBILIZATION

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ABSTRACT

Bacterial cellulose (BC), a microbial polysaccharide, has chemically equivalent structure to plant cellulose with unbranched pellicle structure of only glucose monomers. Due to the unique nanostructure, BC has great potential in enzyme immobilization. In this study, the effects of different cultivation conditions including rotational speed, initial inoculum concentration and medium pH on the film-like cellulosic biomass formation of Gluconacetobacter xylinus JCM 9730 were examined. The resultant BC films were then studied for its feasibility in the immobilization of lipase, a widely used enzyme in biotechnological and industrial processes including food, pharmaceutical, chemical and paper industries. Results showed that increasing in rotational speed from 0 rpm to 200 rpm converted cellulose-producing cells to non-cellulose-producing ones, leading to a significant decline in BC film formation. The increase in initial inoculum size from 0.01 g/L to 0.1 g/L reduced sugar concentration and surface area of the medium, and therefore inhibiting the formation of film-like cellulosic biomass. In addition, the optimum pH range of Acetobacter species from 5.4 – 6.3 was found not optimal for BC film formation. The highest amount of film-like cellulosic biomass of 19.01 g/L was obtained under static condition (0 rpm) with initial cell concentration of 0.04 g/L and initial pH of 4.0. The BC film samples were then acetylated with acetic anhydride/iodine system to convert the hydroxyl groups to less hydrophilic acetyl groups and were used for lipase immobilization. Results showed that lipase immobilized on acetylated BC still maintained its lipid hydrolytic activity. It can be hence concluded that BC films produced by G. xylinus JCM 9730 were potential for lipase immobilization.

Keywords: *Gluconacetobacter xylinus; Cellulosic biomass; Acetylation; Lipase; Immobilization.*

1. INTRODUCTION

Lipases (triacylglycerol acylhydrolase, E.C. 3.1.1.3) are hydrolases acting on carboxylic ester bonds, converting long-chain triacylglycerols into diacylglycerols, monoacylglycerols, glycerol and fatty acids [2]. Lipases still remain a subject of intensive study due to their broad substrate specificity [3], no specific cofactors required in reactions [2], high thermo-stability [4], being active in the presence of organic solvents [4], and ability of catalyzing different types of reactions including hydrolysis, esterification, intesterification, acidolysis, alcoholysis, aminolysis [2]. Lipases are hence the most popular catalyst used in industrial productions of EPA and DHA from fish oil [5]; in infant formula and bread manufacture [6, 7]; in detergent and pharmaceutical industries [2] and in ethyl acetate, ethyl butyrate, ethyl methyl butyrate synthesis [8]. However, most commercial lipases are difficult to recycle and unstable under operation conditions [9], resulting in a high production cost [3]. These disadvantages could be solved by using immobilized enzymes [10]. Immobilization improves recyclability and shelf-life of enzymes, enhances enzyme stability and activity under different reaction conditions [3, 4, 9, 10].

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Several research works on lipase immobilization have been conducted. Lipases were immobilized on different kinds of supports including inorganic supports (for instance silica, hydroxyapatite, titania) and organic supports (natural origin materials such as chitosan, chitin, alginate, plant cellulose, also the synthetic compounds, mainly polymers) [11, 12]. However, some of these supports are relatively expensive and unavailable such as silica, acrylic resin leading to high immobilization cost [11]. Although plant cellulose is more abundant and inexpensive, it should be unhealthy-chemically treated to obtain pure product. Increasing demand on plant cellulose also leads to increased wood consumption, causing global environmental issue [13].

Beside vascular plants, microorganisms, especially the genera of *Gluconacetobacter*, also produce cellulose with considerably different properties. Many studies reported that bacterial cellulose (BC) does not require extra pretreatment to remove impurities and contaminants including lignin, pectin and hemicellulose in plant cellulose. In addition, due to its high degree of crystallinity, high water holding capacity, high mechanical strength and biocompatibility, BC has been used as a support for cell and enzyme immobilization [14]. For instance, BC from *Acetobacter* spp. and *Komobacter* spp., negative-gram bacterial groups living in fruits and vegetables, had been used as non-toxic and potential carrier for different enzymes such as laccase, glucoamylase, tyrosinase, lipase, peroxidase, glucose oxydase [15-18].

Although BC is the potential material for enzyme immobilization, it is hydrophilic in nature, hence BC cannot easily interact with hydrophobic substrates, particularly substrates of lipases. The hydrophilic property of BC is due to its hydroxyl groups located on cellulose surface [1]. Hence, several reagents were used to modify BC surface including triethylaluminium and triethylboron [19], isopropyl dimethylchlorosilane [20], organic acid [21], glutaraldehyde [22], anhydride acetic [1]. Among those methods, acetylation treatment was commonly used due to its ability in reducing the hygroscopicity and maintaining thermal stability of BC materials [1]. In this method, the hydroxyl groups of BC were replaced by less hydrophilic acetyl groups so that the acetylated BC samples did not lose their transparency as well as their large surface area and high porosity [23 - 25]. It was reported that the features of the acetylated BC surfaces not only enhanced catalytic activity of immobilized lipases, but might also direct lipase molecules towards a particular orientation suitable for their active configuration and enhance the stability of the adsorbed enzyme molecules [26]. Thus, acetylated BC (ABC) could be considered as a potential support for lipase immobilization.

Several factors potentially involved in the film-like cellulosic biomass formation of *Gluconacetobacter* species including rotational speed, initial inoculum concentration, pH medium [27 - 29]. Hence this study aimed to investigate the ability to form BC films of *Gluconoacetobacter xylinus*, a model strain widely used in research and commercial production. The effects of rotational speed, initial inoculum size, and medium pH on the production of BC film were examined. After that, the obtained BC films were acetylated to test for its lipase immobilization capacity.

2. MATERIALS AND METHODS

2.1 Enzyme, microorganisms and culture conditions

Commercial lipase was obtained from Novaco Pharmaceutical Co.Ltd. (Hanoi, Vietnam).

Gluconoacetobacter xylinus (JCM 9730) was provided by Department of Biotechnology, Bach Khoa University (HCMC, Vietnam) [30]. *G. xylinus* was maintained on M1 agar plates at 4 ^{o}C. M1 medium was prepared using coconut water with the chemical composition was as follow: 1.0 % (w/v) sucrose, 0.1 % (w/v) yeast extract, 0.05 % (w/v) KH_2PO_4, 0.05 % (w/v) K_2HPO_4, 0.8 % (w/v) $(NH_4)_2SO_4$; 0.2 % (w/v) $(NH_4)_2HPO_4$; 0.1 % (w/v) $MgSO_4$ [31]. The

pH of medium was adjusted to pH 5.0 with concentrated acetic acid. The preculture was prepared by transferring bacterial colonies from agar plate to 250 mL Erlenmeyer flask containing 100 mL of M1 medium. The preculture was agitated at 200 rpm with a magnetic stirrer (MS-H0810, DLab, USA) at room temperature for 72 hours.

For cellulosic biomass production, cell stock from preculture was harvested and inoculated to 100 mL of M2 medium. M2 was prepared using coconut water with the chemical composition was as follow: 1.0 % (w/v) sucrose, 0.1 % (w/v) yeast extract, 0.8 % (w/v) $(NH_4)_2SO_4$, 0.8 % (w/v) $(NH_4)_2SO_4$, 0.5 % (v/v) concentrated acetic acid [31]. The pH of medium was adjusted to pH 5.0 using acetic acid. All the chemicals used in this research were of analytical grade. All media for bacteria, and apparatus such as pipette tips, Erlenmeyer flasks fitted with cotton plugs, magnetic bars were autoclaved at 121 ^oC for 15 min before use.

2.2 Effects of rotational speed on film-like cellulosic biomass production

The *G. xylinus* cells were inoculated at a concentration of 0.04 g/L in a 250-mL Erlenmeyer flask containing 100 mL of M2 medium (medium pH was 5.0). The cell culture was then incubated at 30 ^oC for 7 days using an incubated shaker (IST-3075R, JeioTech, Korea). The effects of rotational speed on the film-like BC formation were investigated at 0 rpm (static condition), 100 rpm, 150 rpm and 200 rpm. Film-like or gel-like cellulosic biomass were daily collected to determine cellulosic biomass concentration. Suspended cell culture samples were also daily collected to measure the suspended biomass concentration, and remaining total sugar concentration. Each experiment was performed in triplicates.

2.3 Effects of initial inoculum size on film-like cellulosic biomass production

G. xylinus cells were added to a 250-mL Erlenmeyer flask containing 100 mL of M2 medium (pH 5.0). The effects of initial inoculum size on the BC formation was investigated at 0.01 g/L, 0.04 g/L, 0.07 g/L and 0.1 g/L. The cell cultures were incubated at 30 ^oC under static condition (0 rpm) for 7 days in an incubated shaker. Film-like cellulosic biomass and suspended bacterial culture were periodically collected to determine cellulosic and suspended biomass concentrations, and total sugar concentration. Each experiment was performed in triplicates.

2.4 Effects of medium pH on cellulosic biomass production

The experimental design was similar to that of initial inoculum size experiment. The *G. xylinus* cells were inoculated at a concentration of 0.04 g/L in a 250-mL Erlenmeyer flask containing 100 mL of M2 medium with medium pH ranging from 4.0 – 6.0. The cell cultures were incubated at 30 ^oC under static condition (0 rpm) for 7 days.

2.5 Acetylation of BC

Film-like cellulosic biomass floating on the surface of liquid medium with thickness of 1.80 ± 0.08 mm (Figure 1) was collected and cut into round shape with a diameter of 1.7 cm. These sheets were washed with distilled water twice and treated with 0.25 M NaOH at 80 ^oC for 1 hour to lyse the cells. BC pieces were washed again with distilled water two times and then dried at 80 ^oC for 2 hours [1].

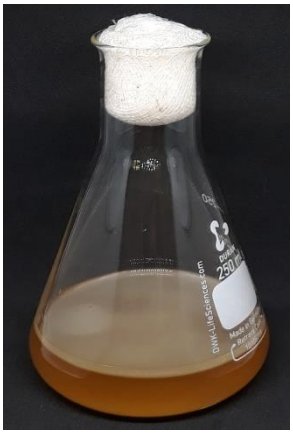

Figure 1*. Film-like cellulosic biomass floating on the medium surface after 7 days of cultivation at static condition.*

400 mg of the dried BC was added to a 100 mL erlen containing a mixture of 20 mL

acetic anhydride and 0.125 mM iodine. The mixture was heated to 80 ^{o}C for 1 h, and then was cooled to room temperature. 50% $Na_2S_2O_3$ was added to the flask until the color of mixture changed from dark brown to colorless, meaning that iodine was converted to iodide [1]. After acetylation, acetylated BC (ABC) samples were washed with 75% ethanol and distilled water for 5 times to remove any remaining by-products and unreacted chemicals. ABC samples were then dried at 60 ^{o}C for 12 h [1]. Dried ABC films were used for lipase immobilization.

2.6 Immobilization of lipase onto ABC

Lipase solution (8 mg/mL) was prepared by adding lipase powder to sodium phosphate buffer (0.05 M, pH 7.0). 5 mg sample of dried ABC films was submerged into 3 mL of lipase solution and gently shaken at 100 rpm at 25 ^{o}C for 2 h in incubated shaker. Immobilized-lipase ABC films was washed three times in sodium phosphate buffer (0.05 M, pH 7.0). The reaction solution and washing buffers were collected after immobilization for determination of residual protein concentration [32, 33].

2.7 Analytical methods

In this study, total *G. xylinus* biomass concentration was calculated as a summation of suspended cell biomass (D1) and cellulosic biomass in the form of film-like or gel-like biomass (D2) (Figure 2). Suspended *G. xylinus* cell growth was monitored by optical density (OD) measurement at 600 nm using an ultraviolet–visible spectrophotometer (UH5300, Hitachi, Japan). The OD_{600} was used to compute the suspended bacterial biomass concentration by the formula:

$$\text{Dry cell weight, D1 (g/L)} = 0.3592 \times OD_{600} \quad (R^2 = 0.9952) \qquad (1)$$

Cellulosic biomass concentration (D2, g/L) was measured by removing the cellulosic materials from the liquid culture and drying at 80 ^{o}C (UF260, Memmert, Germany) until constant weight.

Total sugar concentration was measured using phenol-sulfuric method [34]. pH value was measured using pH meter (HI991003, Hanna, Romani).

The yield ratio, $Y_{BC/S}$ (g/g) of cellulosic biomass to total sugar consumption was calculated using equation (2) [35]:

$$Y_{BC/S}\,(g/g) = \frac{D2}{\text{total sugar consumption}} \qquad (2)$$

Lipase activity was assayed by olive oil emulsion method [33, 36]. One unit of lipase activity (U) was defined as the amount of enzyme that produces 1 μmole of free fatty acid per min under the assay conditions [33]. The immobilization yield and activity yield were calculated as followings [11]:

$$\text{The immobilization yield (\%)} = \frac{A\text{-}B}{A} \times 100 \qquad (3)$$

$$\text{Activity yield (\%)} = \frac{C}{A} \times 100 \qquad (4)$$

with A (U) is the total enzyme activity used for immobilization; B (U) is the unbound enzyme activity; A-B (U) is the theoretical immobilized enzyme activity; and C (U) is the obtained immobilized enzyme activity.

The protein concentration in the solution was determined by the method of Lowry et al. (1951) using egg albumin as standard [37, 38]. The loading percentage of protein was calculated as following [11]:

$$\text{Proteins loading (\%)} = \left(\frac{\text{Amount of adsorbed proteins}}{\text{Initial amount of proteins}}\right) \text{x } 100 \qquad (5)$$

The chemical structures of BC, ABC, and immobilized-lipase ABC were characterized using Fourier-transform infrared spectroscopy (FTIR) (FT/IR-4700, Jasco, Japan) using the attenuated total reflectance attachment (ATR) to investigate the chemical characteristics of BC before and after the acetylation and immobilization. The wavelength region was from 4000 to 400 cm^{-1} with the resolution of 1 cm^{-1}. The FTIR spectra results was showed by Origin software.

All data shown were the means of triplicate measurements. All statistical analyses were carried out using Excel software. All data were expressed as means ± standard deviations.

3. RESULTS AND DISCUSSION

3.1 Effects of rotational speed on film-like cellulosic biomass production

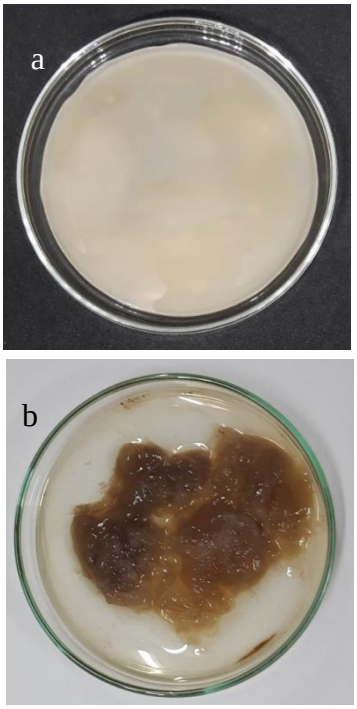

***Figure 2**. Cellulosic biomass in (a) static culture and (b) shaking culture*

It is well-known that cellulosic biomass formation was affected by types of cultivation (static or shaking cultures), initial inoculum, composition of medium and medium pH [39, 40]. Few studies reported that, under shaking condition from 100, 150 and 200 rpm, *G. xilinus* can form gel-like or spherical-like cellulosic biomass, whereas under static condition, it can form film-like biomass [40-42]. Spherical-like or film-like BC was more suitable for enzyme immobilization because it is easier for removal, cleaning and reusing immobilized-enzyme BC particles [25, 43] Hence, effects of rotational speed on *G. xylinus* JCM 9730 growth were conducted to understand its roles on the formation of different types of cellulosic biomass. Results showed that, under static condition, major fraction of *G. xylinus* biomass was in the form of film-like cellulosic biomass floating on the liquid surface (14.79 g/L) (Figure 1, Figure 2a and Figure 3a), whereas the rest was in suspension (0.06 g/L). On the contrary, at rotational speeds of 100 rpm and 200 rpm, cellulosic biomass did form, but in the fibrous form of non-uniform shapes, or so called gel-like BC (Figure 2b). The amount of cellulosic biomass under shaking condition was also lower as compared to that of static condition (3.27 g/L - 100 rpm, 1.56 g/L - 150 rpm, and 1.02 g/L – 200 rpm versus 14.79 g/L). It can be easily seen that, the amount of cellulosic biomass, so-called bacterial cellulose films, obtained under static condition was 4.5 – 14.5 times higher than those of shaking conditions. The obtained results were in contrast with previous study [41]. Mohite et al. (2013) have reported that agitated cultures increased air-liquid surface area, and hence increased cellulose production [41]. However, excessive oxygen supply during agitation could result in a decrease in BC productivity due to loss of substrate by direct oxidation [44, 45]. In addition, shear stress in the shaking culture could convert cellulose-producing cells to non-cellulose-producing mutants [45, 46].

Figure 3c shows that the total sugar biodegradation rate in static culture was much faster than those in shaking cultures (3.89 g/L-day versus 2.92 g/L-day, 3.36 g/L-day and 2.47 g/L-day). In addition, after 7 days, the $Y_{BC/S}$ in static culture was 3.3 – 9.0 times higher than those in shaking cultures (0.54 versus 0.16, 0.07 and 0.06). This could be attributed to the highest total biomass concentration of cells under static condition (14.85 g/L).

To sum up, static condition was suitable for the formation of bacterial cellulosic films of *G. xylinus*. In addition, the high BC film production yield and thin-film structure made the cellulosic films more suitable for the immobilization of lipases. This static condition was hence used in subsequent experiments to produce BC films.

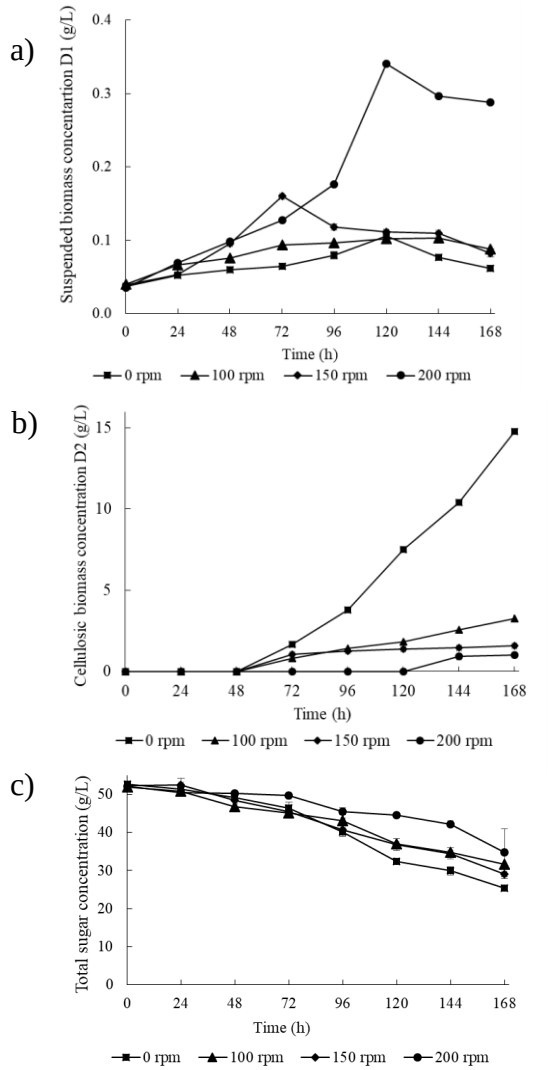

Figure 3. *Effects of rotational speed on (a) suspended cells biomass concentration D1, (b) cellulosic biomass concentration D2, (c) total sugar concentration.*

3.2 Effects of initial inoculum size on BC film production.

Initial cell concentration is one of important factors affecting cellulosic biomass formation. According to Hu et al. (2013), a high cell concentration and adequate food supply might enhance cellulosic biomass production rate due to a fast aggregation of cellulose fibers on the culture surface [40]. Results showed that at highest initial cell concentration (0.1 g/L), most of the cells were in suspension (Figure 4a), and no cellulosic biomass were formed (Figure 4b). The concentration of suspended cells significantly increased at day 3 of cultivation (0.72 g/L), more 1.3 – 24.8 times higher than those at other initial inocula. However, *G. xylinus* cells in this culture (0.1 g/L of inoculum size) did not completely consume the total sugar content in medium (Figure 4c), proving that the dissolved oxygen in the medium had been exhausted due to high initial cell concentration. Hence, it can be concluded that *G. xylinus* can not ultilize sugar under anaerobic condition to produce BC.

Results in Figure 4 also show that at lowest initial cell concentration of 0.01 g/L, the suspended cell and cellulosic biomass concentrations were very low. Hence, at low initial cell concentration, the high initial concentration of sugars in the medium could have inhibited the cell growth.

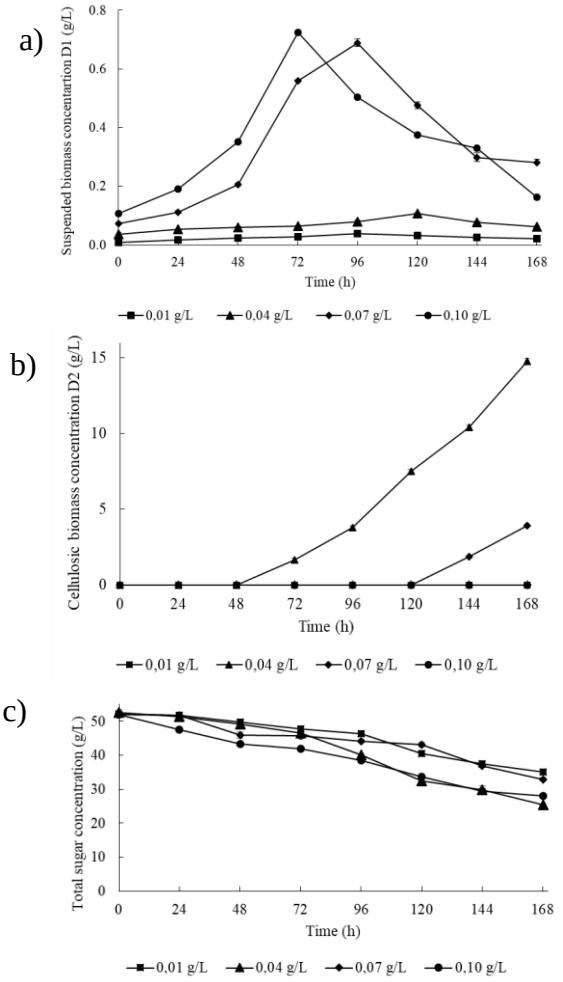

Figure 4*. Effects of initial inoculum size on (a) suspended cells biomass concentration D1, (b) cellulosic biomass concentration D2, (c) total sugar concentration.*

On the other hand, at 0.04 g/L and 0.07 g/L initial inoculum sizes, film-like cellulosic biomass were synthesized with final concentrations of 14.79 g/L and 3.91 g/L, respectively. Results of the total sugar consumption showed that slower sugar consumption rate at 0.07 g/L of initial inoculum size (2.72 g/L-day) could also be due to the exhaustion of dissolved oxygen in the culture. Therefore, the increasing of initial biomass concentration from 0.04 g/L to 0.1 g/L did not enhance cellulosic biomass production. At higher initial biomass concentrations (0.07 g/L and 0.1 g/L), bacterial cells would compete for the sugars in the culture, which may lower cellulosic biomass productivity.

To sum up, 0.04 g/L initial inoculum size sample showed highest sugar consumption rate and highest cellulosic biomass production. Initial biomass concentration of 0.04 g/L was hence profitable for the formation of film-like bacterial cellulosic biomass, and hence was used in the next experiment.

3.3 Effects of medium pH on cellulosic biomass production.

Medium pH plays an important role on the structure and permeability of the cell membrane [47]. Many studies reported various optimum pH ranges for BC production of *Gluconacetobacter* species, such as 4.5 – 5.5 [47]; 4.0 – 5.0 [48]; 4.0 – 6.0 [41, 46, 49]; 4.0 – 7.0 [50]; 4.0 – 4.5 [51]. In addition, it has been reported that *G.xylinus* did not produced cellulosic biomass at pH 3.5 because of complete inhibition by the low pH [28, 47]. Hence, in this study, effects of initial medium pH on film-like biomass production were investigated at pH values of 4.0, 5.0, and 6.0. Results on Figure 5b show that, the amount of cellulosic biomass at pH 4.0 after 7 days of cultivation was 19.01 g/L, 1.3 – 2.0 times greater than those of pH 5.0 sample (14.79 g/L) and pH 6.0 sample (9.52 g/L). It is clear that beside acting as an energy source and cellulose precursor, glucose (from coconut water) was also actively converted by membrane-bound *Gluconoacetobacter* dehydrogenases to (keto)gluconic acids [28, 52]. That conversion decreased pH of medium, adversely affecting bacterial growth rate and cellulose synthesis [27, 28, 52, 53]. It should be noted that M2 medium was added with glacial acetic acid to adjust the pH. Not only acting as a pH monitorer, acetic acid was also a substrate for *Acetobacter* spp., which was ultimately oxidized to CO_2 and water. That oxidation process generated acetic acid-derived ATP, saving part of the D-glucose in medium for being incorporated into cellulose [39, 52]. The catabolism of acetic acid simultaneously leads to an increase in medium pH, which could counteract the pH decrease caused by (keto)gluconic acids formation (pKa of acid acetic is 4.75) [52].

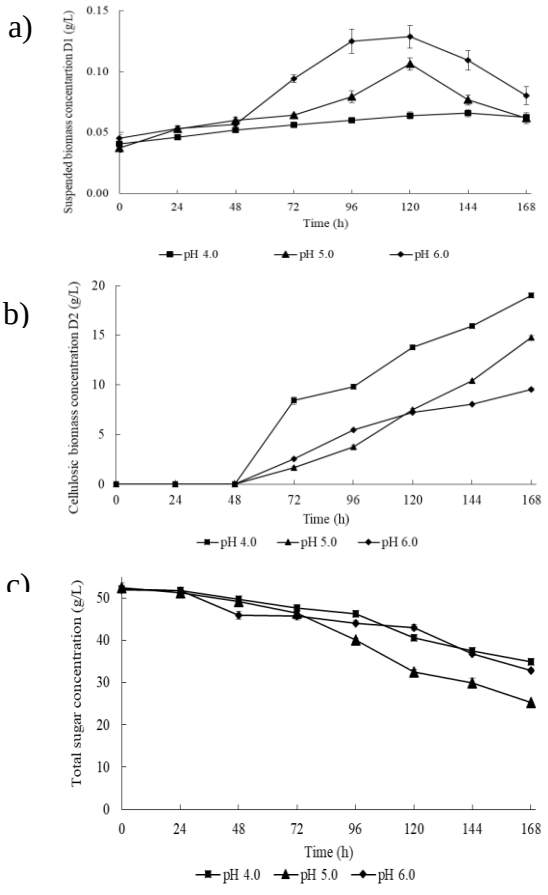

Figure 5*. Effects of medium pH on (a) suspended cells biomass concentration D1, (b) cellulosic biomass concentration D2, (c) total sugar concentration.*

It was also reported that the optimum pH for *Acetobacter* spp. growth was between 5.4 - 6.3 [53], this might not be the same optimum pH range for cellulose synthesis [47]. Results in Figure 5a showed that suspended biomass concentration of *G. xylinus* after 5 days in pH 6.0 was more 1.2 – 2.2 times higher than the others (0.13 g/L versus 0.11 g/L and 0.06 g/L). In contrast, the $Y_{BC/S}$ was gradually enhanced when decreasing initial medium pH. Specifically, at day 7, the yield of cellulosic biomass production to the total sugar consumption in initial pH 4.0 condition was 2.1 – 2.2 times higher than that in initial pH 5.0 and 6.0 conditions (1.11 g/g versus 0.54 g/g and 0.50 g/g).

Results of this experiment confirm at initial medium pH value of 4.0, cellulosic biomass were synthesized with film-like form at highest concentration, profitable for enzyme immobilization.

3.4 Acetylation of BC film

Results in current work showed that under static cultivation condition at 30 ºC with 0.04 g/L of initial inoculum size and pH 4, highest film-like cellulosic biomass was obtained. The BC biomass was then collected for acetylation treatment. The optimum conditions for BC acetylation in previous study was used to examine the efficiency of this treatment method (see Part 2.5). The acetylated BC samples were then used to immobilize lipases. The acetylation mechanism is shown in Figure 6. Firstly, in the presence of alcohol groups, iodine activates the carbon of carbonyl groups of acetic anhydride. After that, the oxygen of R-OH attacks the carbon of carbonyl groups of acetic anhydride to form sp^3 hybridization. The acetylated reaction starts when the acyl carbon center of acetic anhydride is nucleophilic attacked by lone pair of the hydroxyl group to from free iodine. Then, a saturated sodium thiosulfate solution is added to remove all the free iodine [1]. FT-IR analysis of BC films was performed to detect the chemical groups present in their chemical structure. The infrared spectra of BC, ACB and immobilized-lipase ABC films are shown in Figure 6.

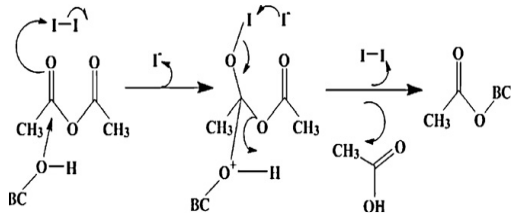

***Figure 6**. The mechanism of BC-acetylated reaction using iodine as the catalyst* [1].

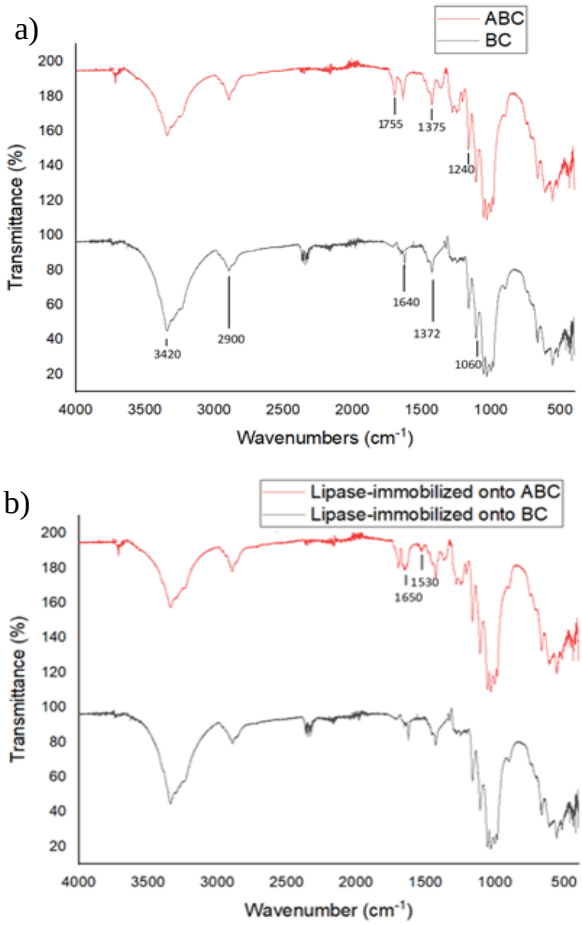

***Figure 7**. Infrared spectra of (a) untreated BC, and ABC, (b) BC and ABC with immobilized lipases.*

As can be seen in Figure 7, BC films present characteristic absorption bands of cellulose in FTIR such as 3700 – 3000 cm^{-1} (O-H), 2980 – 2800 cm^{-1} (C-H), 1640 cm^{-1} (H_2O molecules), 1500 – 800 cm^{-1} (C-H, O-H, C-O and C-O-C) [56-59]. The characteristic of acetyl groups were identified in FTIR spectra of ABC fibers such as 1755 - 1720 cm^{-1} (C=O), 1372 - 1369 cm^{-1} (C-H), 1240 - 1229

cm^{-1} (C-O) [1, 24, 56, 57, 60-62]. Moreover, infrared spectra of ABC sample also show the decreased absorption intensity of the band located at around 3700 – 3000 cm^{-1} as compared with BC sample. It can be concluded that the hydroxyl groups of BC were substituted by acetyl groups in ABC [1, 24, 57]. Besides, the absorption bands at 1840 – 1760 cm^{-1} and 1700 cm^{-1} were not found, indicated that ABC samples was also free of acetic anhydride and acetic acid [1, 56, 63, 64].

Results of Figure 7b also shows that the absorption bands at 1530 cm^{-1} and 1650 cm^{-1} were also detected on the infrared spectrum of ABC immobilized with lipases, probably due to the presence of amide I and amide II bands [56]. This result confirmed the presence of protein molecules (lipases), on the surface of ABC supports. To confirm the presence of immobilized enzyme, lipid hydrolytic activity of immobilized-lipase ABC was investigated.

3.5 Lipid hydrolytic activity of lipase immobilized on ABC.

The hydrolytic activity of lipases immobilized on ABC and BC is presented in Table 1. Results show that lipases immobilized onto BC support did not show any catalytic activity. In contrast, the immobilization yield, activity yield, protein loadings of lipase immobilized onto ABC were significantly higher those of untreated BC. These results further confirmed that lipase molecules were immobilized on ABC support and still preserved their catalytic activity as compared to lipases immobilized on untreated BC. The results also indicated that ABC could be more easily interact with hydrophobic substrates lipases, leading to significant enhancement in lipid hydrolytic activity.

Table 1*. Immobilization yield of lipase-immobilized on BC and ABC*

Support	BC	ABC
Immobilization yield (%)	0.49 ± 0.07	6.97 ± 0.14
Protein loading (%)	0.45 ± 0.56	5.43 ± 0.98
Activity yield (%)	0.38 ± 0.66	2.32 ± 1.15
Specific activity (U/mg protein)	0.00 ± 0.00	0.76 ± 0.44

4. CONCLUSION

The formation of film-like cellulosic biomass by was investigated under the influences of rotational speed, initial inoculum size and pH medium. The highest amount of film-like cellulosic biomass of 19.01 g/L was obtained under static condition (0 rpm) with initial cell concentration of 0.04 g/L and initial pH of 4.0. This cultivation condition allowed the formation of cellulosic films which were more suitable for the immobilization of lipases. The BC films were acetylated by acetic anhydride with iodine as a catalyst to immobilize lipase. Lipase immobilized on ABC showed significantly higher catalytic activity in lipid hydrolysis as compared to lipase immobilized on BC. Therefore, acetylated BC is the potential support for the immobilization of lipases.

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